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Research Article

Keywords: antibacterial activity, clinical isolates, Gram-positive, MSSA, MRSA, ozenoxacin, skin and soft tissue infections

Posted Date: September 29th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10859393/v1>

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Additional Declarations: No competing interests reported.

***In-vitro* evaluation of Ozenoxacin and other Antibiotics against *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from Skin and Soft Tissue Infections**

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41 **Abstract**

42 **Background** *Staphylococcus aureus* and *Streptococcus pyogenes* are major causative bacteria responsible
43 for skin and soft-tissue infections (SSTIs) such as impetigo. Increasing resistance to commonly used
44 topical antibiotics necessitates evaluation of newer agents for the treatment of skin infections. Ozenoxacin, a
45 novel non-fluorinated topical quinolone, has shown promise, exhibiting potent activity against a wide
46 range of pathogens, including methicillin-resistant *Staphylococcus* (MRSA) and *Streptococcus*
47 *pyogenes*.

48 **Methods** The present study compared the *in vitro* activity of ozenoxacin and comparator agents
49 against clinical isolates of *Staphylococcus aureus* and *Streptococcus pyogenes* from multiple sources
50 including skin and soft-tissue, wound, abscess, and blood. Ozenoxacin was assessed for *in vitro*
51 antimicrobial activity against 109 methicillin-susceptible (MSSA), methicillin-resistant *S. aureus* (MRSA),
52 and 24 *Streptococcus pyogenes* isolates by broth microdilution method recommended by the Clinical and
53 Laboratory Standards Institute (CLSI).

54 **Results** Ozenoxacin demonstrated potent *in-vitro* activity against all 109 *S. aureus* (MIC_{50/90} = 0.125/0.5
55 µg/ml) and 24 *S. pyogenes* (MIC_{50/90} = 0.015/0.03 µg/ml) strains.

56 In contrast, higher MICs were observed for fusidic acid and mupirocin among a subset of *S. aureus*
57 isolates. A comparison of MIC₉₀ values demonstrated that ozenoxacin (0.5 µg/ml) was more active
58 against
59 *S. aureus* isolates than 8 of the 9 comparator agents tested including vancomycin and linezolid (MIC₉₀ =
59 2 & 4 µg/ml) respectively.

60 **Conclusions** *In vitro* studies of ozenoxacin showed potency against staphylococci and streptococci
61 including resistant *S. aureus* strains. These findings support its role as an effective first-in-class quinolone
62 topical therapeutic option in the management of various SSTIs.

61
62 **Keywords** antibacterial activity, clinical isolates, Gram-positive, MSSA, MRSA, ozenoxacin, skin
63 and soft tissue infections
64

65 **Background**

66 The principal barrier against microbial invasion is the skin. It constantly interacts with the external
67 environment and is colonized with a diverse population of microbes. Most of the colonizing flora
68 consists of bacteria. To help organize the distribution of flora, one can divide the body into two halves
69 at the waistline. The typical organisms that colonize the skin above the waist are usually Gram-
70 positive species such as *Staphylococcus epidermidis*, *Staphylococcus aureus* (*S. aureus*), and
71 *Streptococcus pyogenes* and *Corynebacterium* species. *S. aureus* and *S. pyogenes* species are
72 particularly significant because they contribute to a majority of skin and soft tissue infections (SSTIs)

73 (1). *Staphylococcus aureus* (*S. aureus*) is both a commensal bacterium that is persistently or
74 transiently present in up to 80% of healthy individuals and a Human pathogen causing a wide
75 spectrum of clinical infections in humans, ranging from superficial SSTIs to deeply invasive life-
76 threatening bacteraemia and endocarditis.

77 Skin and soft tissue infections are clinical entities of variable presentation, etiology and severity that
78 involve a broad range of conditions involving the dermis and subcutaneous tissue, extending from
79 minor superficial processes/ infections such as pyoderma to deeper tissue involvement such as
80 necrotizing fasciitis with potentially fatal complications that demand immediate attention (2).

81 Impetigo is a highly contagious bacterial superficial skin infection, that primarily affects children
82 between 2 to 5 years of age, however, individuals of any age can be susceptible to this bacterial
83 condition (3). It is classified under the broader category of SSTIs which represent a significant
84 proportion of community- and hospital-acquired infections worldwide. Impetigo though common, is
85 often self-limiting, in which systemic symptoms are rare and is an underdiagnosed important
86 pediatric dermatological health problem all over the world, that, if left untreated, can have serious
87 consequences (4).

88 **Evolving Microbiology and Clinical Implications**

89 The most common pathogens implicated in impetigo are *S. aureus* (including methicillin-resistant *S.*
90 *aureus* (MRSA) and *S. pyogenes*. Impetigo is a bacterial infection where the causative organism is
91 not routinely identified, however an epidemiological shift from *S. pyogenes* to *S. aureus* has been
92 observed (5). Infection with *S. aureus* or *S. pyogenes* causes the nonbullous type of impetigo which
93 occurs in around 70% of cases, whereas *S. aureus* exclusively causes bullous impetigo, with the
94 production of exfoliative toxins (6). This epidemiologic shift has important clinical consequences
95 since *S. aureus* carries a large repertoire of virulence determinants in conjunction with different
96 antibiotic resistance phenotypes more than *S. pyogenes*. Previously restricted to the hospital
97 environment, *S. aureus* is now more widespread outside healthcare facilities and SSTIs caused by
98 community-associated MRSA (CA-MRSA) have been increasing over the last decade and a serious
99 problem worldwide (7).

100 Topically applied antibiotics, such as mupirocin and fusidic acid, have been the mainstay/first-line
101 of treatment for superficial SSTIs and localized impetigo, although retapamulin is a more recent
102 alternative (8). The development of new antibiotics represents a current need, considering the
103 increase in multidrug-resistant pathogens, such as MRSA and mupirocin-resistant or quinolone-
104 resistant *Staph aureus*, raising concerns regarding their continued effectiveness (9). Moreover, fusidic
105 acid monotherapy, specifically topical formulations, has been strongly linked to the emergence of
106 fusidic acid resistance among either MRSA or methicillin- susceptible *Staph aureus* (MSSA) (10).

This increasing resistance complicates treatment options and highlights the need for newer topical antimicrobials with improved efficacy and a lower propensity for resistance development (11). The most recent topical option ozenoxacin a novel nonfluorinated quinolone with bactericidal activity, is approved in the USA, Canada and 12 EU countries as a 1% cream formulation for treatment of impetigo in children and adults and in Japan as a 2% lotion formulation for treatment of superficial skin infections and acne. Approval of ozenoxacin was granted based on demonstrable clinical benefit in two large Phase III trials of impetigo involving adults and children from 2 months of age (8). Rapid eradication of bacterial load is important in impetigo to hasten symptom resolution and limit person-to-person transmission of infection. Usually, gyrase inhibitors are more active against DNA gyrase than topoisomerase, while quinolones show more inhibitory potency against Topoisomerase IV than that of Gyr B (12). Interestingly, ozenoxacin's dual inhibitory activity against DNA gyrase (topoisomerase II), and topoisomerase IV, required for bacterial DNA replication will pose lower risks of resistance development than single-target inhibitors (13). Lack of a fluorine atom at the C-6 position theoretically has less phototoxicity potential than traditional fluoroquinolones which contributes to its favourable safety profile and reduced systemic absorption, and is therefore considered suitable for pediatric use. Ozenoxacin has potent antibacterial activity against *S. aureus* (MSSA, MRSA) and *S. pyogenes* irrespective of their levofloxacin susceptibility status. The present study aimed to evaluate the *in-vitro* antibacterial activity of ozenoxacin against clinical isolates of *S. aureus* (including MRSA and MSSA) and *S. pyogenes* obtained from SSTIs and to compare its activity with commonly used topical agents and selected systemic antibiotics.

Study Rationale and Objectives

In view of the increasing incidence of resistant pathogens, *in vitro* assessment of the activity of ozenoxacin against recent clinical isolates is important. This study aimed to:

- 131 1. Assessment of the *in vitro* antibacterial activity of ozenoxacin against clinical isolates of *S. aureus*
132 (including MRSA) and *S. pyogenes* obtained from patients with skin infections and other sources.
133 2. Compare the efficacy of ozenoxacin with that of other commonly used topical antimicrobial agents,
134 and relevant systemic antibiotics.
135 3. To assess the susceptibility patterns of these clinical isolates, including the presence of resistance
136 to existing topical treatments.

137 **Material and Methods**

138 **Study Design and Clinical Isolates Collection**

139 The study was conducted in the Bacteriology lab in Centre for Drug Design Discovery and
140 Development (C4D) at SRM University Delhi NCR, Sonipat, Haryana. Ethical approval was obtained
141 from the Institutional Ethics Committee. This study involved bacterial isolates and the isolates were
142 collected from the microbiology laboratories. This was a laboratory-based, cross-sectional *in-vitro*
143 study designed to evaluate the antibacterial activity of ozenoxacin against Gram-positive clinical
144 isolates associated with skin and soft tissue infections and other sources.

145 A total of 133 clinical isolates, including *Staphylococcus aureus* (n=109), and *S. pyogenes* (n=24),
146 were included in this study. These isolates include American Type Culture Collection (ATCC;
147 Manassas, VA, USA) strains, and clinical isolates obtained from Indian hospitals were also used in
148 *in vitro* screening. Gram-positive bacteria included in the study as principal pathogens were
149 *Staphylococcus aureus* clinical strains obtained from the culture bank at C4D. The culture bank
150 comprises well-characterized clinical isolates recovered from specimens submitted by tertiary care
151 hospitals and accredited diagnostic laboratories. Isolates were sourced from patients and only one
152 isolate per patient was included to avoid duplication (non-duplicate) in the study. A total of 109 *S.*
153 *aureus* and 24 *Streptococcus pyogenes* clinical isolates were profiled for their *in-vitro* susceptibility
154 to ozenoxacin and comparator antimicrobial agents.

155 Each isolate was identified at the referring hospital/laboratory and added to the culture collection in
156 C4D, SRM University, Sonapat. Each isolate was accompanied by relevant data, including genus and
157 species name. Upon receipt, isolates were sub-cultured on to blood agar to ensure viability and purity.
158 Methicillin resistance among *S. aureus* isolates was determined using Cefoxitin susceptibility testing
159 in accordance with CLSI guidelines, and isolates were categorized as methicillin-resistant (MRSA)
160 or methicillin-sensitive (MSSA). Strains were stored at -80°C in 20% glycerol in Trypticase soy
161 broth (TSB) (Becton, Dickinson and Company, Cockeysville, MD).

162 **Media, reagents, and antimicrobial agents**

Investigational Product

The investigational product i.e. ozenoxacin IP (Batch No. WS/EGY/22?01) and comparator drugs/ antibiotics fusidic acid (Batch No. L12A424901), mupirocin (Batch No. L324223996), amoxycillin clavulanic acid (ACV-1123228) and clindamycin (P-003-CN20230603) were kindly provided by Walter Bushnell Enterprises Private Limited. Remaining antibiotics i.e. vancomycin, linezolid, levofloxacin, neomycin and cefoxitin were procured from Sigma- Aldrich Co. LLC, USA, and Hi Media, India.-All media and reagents were purchased from Becton Dickinson and Company and HI Media.

In vitro Antimicrobial Susceptibility Testing

Minimum inhibitory concentration (MIC) of ozenoxacin and other comparator drugs was determined against Gram-positive bacterial strains using broth microdilution method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines. Susceptibility was determined using breakpoints set by the CLSI, except that British Society for Antimicrobial Chemotherapy (BSAC) breakpoints were used for fusidic acid and mupirocin against staphylococci. Concentrations of antibacterial agent tested for the isolates are shown in Table1. Quality control was ensured using *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619 and *Escherichia coli* ATCC 25922 as standard reference strains recommended by CLSI. All quality control results were within the ranges specified by the CLSI.

Table 1 Concentrations of antibacterial agents tested for *S. aureus* and *S. pyogenes* strains

Antibacterial agents	Antibacterial range tested (µg/ml)	
	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>
Ozenoxacin	0.004–2	0.004–2
Fusidic Acid	0.03–16/ 0.125–128	0.03–16
Mupirocin	0.03–16/ 0.125–128	0.03–16
Neomycin	0.006–32	0.03–16
Amoxycillin clavulanic acid	0.125–64	0.125–64
Clindamycin	0.06–32	0.03–16
Linezolid	0.03–16	0.03–16
Levofloxacin	0.006–32	0.03–16
Vancomycin	0.03–16	0.03–16
Cefoxitin	0.125–64	Not tested

Statistical Analysis

To formally assess whether ozenoxacin has lower anti-microbial activity thresholds than the comparator drugs, the MIC values for each antibiotic were first log₂-transformed to put them on a roughly linear scale.

185 For each antibiotic we calculated the median $\log_2$ (MIC) and interquartile range (IQR). Pairwise two-
186 sided Mann–Whitney U tests were performed in IBM SPSS Statistics (v.25) to compare the
187 distribution of MIC values for ozenoxacin versus each other antibiotic. Effect sizes were calculated
188 as $r = Z/\sqrt{N}$, where Z is the standardized test statistic and N is the total sample size. To control the
189 familywise error rate across the nine tests, p-values were Bonferroni-adjusted. A Bonferroni-adjusted
190 $p < 0.0056$ was considered statistically significant.

191 **Results**

192 **Comparative Antimicrobial activity against Gram-positive bacteria**

193 Ozenoxacin is a novel non-fluorinated quinolone that is being developed for topical use. In this study
194 the *in vitro* activity of ozenoxacin was evaluated against Gram-positive isolates primarily *S. aureus*.
195 In addition, ozenoxacin was also evaluated against *Streptococci pyogenes* clinical strains to see the
196 trend and expand with more strains in future studies. The *in vitro* activities of ozenoxacin against
197 Gram-positive bacteria such as *S. aureus* and *S. pyogenes* are summarized in Table 2

198 ***In vitro* Activity of Ozenoxacin against Gram-positive Pathogens**

199 ***Staphylococci aureus***

200 Ozenoxacin exhibited good *in vitro* activity against all 109 *S. aureus* strains ($MIC_{50/90} = 0.125/0.5$
201 $\mu\text{g/ml}$) respectively (Table 2). Ozenoxacin retained activity against *S. aureus* including MRSA,
202 regardless of methicillin resistance or levofloxacin nonsusceptibility, indicating that its antibacterial
203 activity was largely unaffected by these resistance mechanisms.

Table 2 Minimum inhibitory concentration (MIC) values of Ozenoxacin shown by Methicillin resistance / susceptibility and/or Levofloxacin susceptibility/nonsusceptibility for Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*)

Organism	Levofloxacin susceptibility	n	Ozenoxacin MIC (µg/ml)		
			MIC ₅₀	MIC ₉₀	Range
<i>Staphylococcus aureus</i>	All	109	0.125	0.5	0.004–2
	Levofloxacin non-susceptible	95	0.125	0.5	0.015–2
	Levofloxacin susceptible	14	0.006	0.125	0.004–0.25
Methicillin-resistant <i>S. aureus</i> (MRSA)	All	85	0.125	0.5	0.004–2
	Levofloxacin Non-susceptible	80	0.125	0.5	0.015–2
	Levofloxacin susceptible	5	0.004	0.015	0.004–0.015
Methicillin-susceptible <i>S. aureus</i> (MSSA)	All	24	0.125	0.25	0.004–0.5
	Levofloxacin non-susceptible	15	0.125	0.25	0.03–0.05
	Levofloxacin susceptible	9	0.008	0.015	0.004–0.25
β-haemolytic streptococci <i>S. pyogenes</i>	All	24	0.015	0.03	0.004–0.125
	Levofloxacin non-susceptible	1	0.125	0.125	0.125
	Levofloxacin susceptible	23	0.015	0.03	0.004–0.03

A comparison of MIC₉₀ values demonstrated that ozenoxacin (0.5 µg/ml) was more active against *S. aureus* isolates than 8 of the 9 comparator agents tested including vancomycin and linezolid (MIC₉₀ =2 & 4 µg/ml); fusidic acid (MIC₉₀ =8 µg/ml); levofloxacin, neomycin, amoxicillin-clavulanate and clindamycin (64 µg /ml); and cefoxitin 128 µg/ml). Mupirocin was the only comparator tested that had a MIC₉₀ value (0.5 µg/ml) equivalent to that of ozenoxacin (Table 3).

212 **Table 3** Comparative minimum inhibitory concentration data of Ozenoxacin and other antibacterial
 213 agents for Gram-positive isolates from skin and soft tissue infections

Organisms (no of strains)	MIC	Antimicrobial agent(s)									
	µg/ml	OZN	FUS	MUP	VAN	LZD	LVX	NEO	AMC	CLI	CFX
<i>Staphylococcus aureus</i> (n=109)	MIC ₅₀	0.125	0.25	0.25	1	2	8	4	16	0.125	32
	MIC ₉₀	0.5	8	0.5	2	4	64	64	64	64	128
<i>Streptococcus pyogenes</i> (n = 24)	MIC ₅₀	0.015	8	0.125	0.5	1	0.5	16	0.03	0.03	ND
	MIC ₉₀	0.03	8	0.25	0.5	1	0.5	>16	0.125	4	

AMC: Amoxicillin/Clavulanate; FUS: Fusidic acid; LVX: Levofloxacin; LZD: Linezolid; MUP: Mupirocin; NEO: Neomycin; OZN: Ozenoxacin; VAN: Vancomycin. MIC: Minimum inhibitory concentration; ND: Not done

217 **Activity of Ozenoxacin against *Streptococcus pyogenes***

218 Ozenoxacin exhibited low MIC values (MIC_{50/90}=0.015/0.03 µg/ml) against *S. pyogenes*, indicating
 219 high potency. Ozenoxacin was highly active against levofloxacin non susceptible/susceptible
 220 *S. pyogenes* with a MIC₉₀ of 0.03µg/ml (Table2). Against *S. pyogenes*, ozenoxacin had the lowest
 221 MIC₉₀= 0.03 µg/ml of all other antibacterial agents tested. Whereas mupirocin showed two dilutions
 222 higher MIC 90 0.25 µg/ml and another topical agent fuscidic acid had much higher MIC₉₀ 8 µg /ml in
 223 comparison to ozenoxacin. Neomycin had least activity against *S. pyogenes* MIC₉₀ >16 µg /ml.
 224 MIC 90 values for other comparator agents were higher than that of ozenoxacin: 0.125 µg /ml for
 225 amoxicillin- clavulanic acid: 0.5 µg /ml for vancomycin and levofloxacin and 1µg/ml for linezolid (Table
 3).

226 **Statistical Analysis**

227 The results also indicate that the mean MIC of ozenoxacin is lower than of all other antibiotics
 228 considered for analysis. The median log₂(MIC) for ozenoxacin was -3.00 (IQR -4.06 to -2.00) across
 229 all isolates. Comparatively, the other antibiotics had higher median log₂(MIC) values, indicating
 230 lower potency (higher MIC): e.g. fusidic acid -2.00 (-3.00 to -1.00), mupirocin -2.00 (-2.00 to -
 231 1.00), vancomycin 0.00 (-1.00 to 0.00), levofloxacin 3.00 (2.00 to 5.00), etc.
 232 Pairwise Mann–Whitney tests showed that ozenoxacin MIC values were significantly lower than
 233 those of all other studied antibiotics except Clindamycin. For example, against cefoxitin the U
 234 statistic was 2.0 (Z = -12.753, p<0.001), and the rank-biserial effect size was r = 0.86. Similarly, the
 235 comparisons versus linezolid (U=236.0, Z = -12.251, p<0.001, r=0.83), amoxicillin–clavulanate
 236 (U=234.0, Z = -12.255, p<0.001, r=0.83), and vancomycin (U=1037.5, Z = -10.529, p<0.001,
 237 r=0.71) all yielded highly significant differences (Bonferroni-corrected p<0.001). In contrast,

238 clindamycin had an almost identical median MIC to ozenoxacin (both –3.00) and the test was non-
239 significant, indicating no appreciable difference in potency (Table 4).

240 **Table 4** Statistical output using Pairwise Mann–Whitney U tests comparing Ozenoxacin
241 vs. each antibiotic (n=109 each group) against *S. aureus*

Comparator Antibiotics	Mann-Whitney U test	Z	r	p-value*
Fusidic acid	3850.5	–4.488	0.3	< 0.001
Mupirocin	3266.5	–5.743	0.39	< 0.001
Vancomycin	1037.5	– 10.5 29	0.71	< 0.001
Linezolid	236	– 12.251	0.83	< 0.001
Levofloxacin	506.5	– 11.6 70	0.79	< 0.001
Neomycin	570.5	– 11.5 32	0.78	< 0.001
Amox clavulanate	234	– 12.255	0.83	< 0.001
Clindamycin	5265.5	–1.450	0.1	Not significant
Cefoxitin	2	–12.753	0.86	< 0.001

r is the effect size ($Z/\sqrt{N}$). p-values are two-sided; p_{adj} = Bonferroni-corrected.

***Significant (Bonferroni-adjusted) p-values are in bold.**

242 Because Mann–Whitney tests rank-based differences rather than assuming normality, these results
243 are robust to outliers and non-normal MIC distributions. The use of Bonferroni correction ensured a
244 conservative control of type I error across multiple tests. Even under this strict criterion, ozenoxacin
245 was significantly more potent than virtually all comparators. These findings support the interpretation
246 that ozenoxacin exhibits significantly stronger antimicrobial activity (lower MIC) relative to the other
247 antibiotics in this study.

248 Discussion

249 Ozenoxacin, a novel nonfluorinated quinolone with bactericidal activity, is approved in the USA,
250 Canada and 12 EU countries as a 1% cream formulation for treatment of impetigo and in Japan as a
251 2% lotion formulation for treatment of superficial skin infections and acne. During development of
252 ozenoxacin, surveillance studies compared its *in vitro* antibacterial activity with that of other topical
253 and systemic antibacterial agents (8). The current comparative study examined the antibacterial
254 activity of ozenoxacin in Gram-positive clinical isolates derived mainly from SSTIs, RTIs and UTIs,
255 which were stratified according to their methicillin and levofloxacin susceptibility status. The
256 antibacterial activity of ozenoxacin was compared with that of 9 other antibacterial agents against
257 133 Gram-positive isolates.

The results of this study demonstrate that ozenoxacin possesses potent *in-vitro* antibacterial activity against Gram-positive clinical isolates from SSTIs, including MRSA and *S. pyogenes*. This is in line with results of previously published comparative studies wherein ozenoxacin was shown to be a potent antibacterial agent against Gram-positive (Staphylococci and Streptococci) isolates (8).

The low MIC values observed indicate superior activity compared to commonly used topical antibiotics such as mupirocin and fusidic acid. Statistical significance results are observed with p-value of (<.0001) for all comparator antibiotics except clindamycin against the MSSA and MRSA clinical isolates. Resistance to mupirocin and fusidic acid has been increasingly reported, particularly among *S. aureus* isolates. In this study, elevated MICs for these agents were observed in a subset of isolates, highlighting the clinical relevance of alternative topical therapies.

Ozenoxacin's dual inhibition of DNA gyrase and topoisomerase IV, along with its non-fluorinated structure, may contribute to its enhanced activity and reduced potential for resistance development. The observed efficacy against MRSA is particularly significant, given the limited topical options available for such infections (14). Although linezolid and levofloxacin demonstrated good *in-vitro* activity, their systemic use and potential adverse effects limit their applicability as first-line agents for superficial SSTIs. Therefore, there is a high unmet medical need for a new class of anti-MRSA antibiotic to overcome the limitations of SOC and fight against emerging resistance (12).

Limitations

This study was limited to *in-vitro* analysis and did not assess clinical outcomes. Additionally, pharmacokinetic and pharmacodynamic correlations were not evaluated. Further clinical studies are warranted to corroborate these findings.

Conclusion

In this study, the *in vitro* activity of ozenoxacin against Gram-positive clinical isolates is compared with a panel of commonly used antibacterial agents and ozenoxacin was shown to be a potent antibacterial agent against *S. aureus* and *S. pyogenes* isolates. The excellent activity of ozenoxacin in staphylococcal and streptococcal spp. including methicillin and quinolone-resistant strains most commonly associated with childhood impetigo suggests that ozenoxacin may be a valuable alternative to other topical antibiotics in the topical management of SSTIs and underscores its importance in antimicrobial stewardship initiatives.

287 **Abbreviations**

288 SSTIs Skin and soft-tissue infections

289 MRSA Methicillin resistant *S. aureus*

290 MSSA Methicillin-susceptible *S. aureus*

291 MIC Minimum inhibitory concentration

292 **Declarations**

293 **Ethics approval and consent to participate**

294 This was a retrospective, laboratory-based cross-sectional *in-vitro* study designed to evaluate the
295 antibacterial activity of ozenoxacin against Gram-positive clinical isolates. The clinical isolates were
296 collected from the microbiological laboratories of the hospital network in Delhi-NCR.

297 The study was approved by the Ethics Committee (Approval No. SRMUH/UEC/2026/001, dated 23 June 2026
298 of the Institutional Review Board (Registration No. SRMU020520243282) of SRM University, Delhi-NCR,
299 Sonapat, Haryana, India. Requirement for individual informed consent was waived because archived,
300 de-identified bacterial isolates were used, with no personally identifiable information (PII) and protected health
301 information (PHI) belonging to the patient from whom they were isolated and no direct patient recruitment.

302 **Consent for publication**

303 All authors have read and agreed to the published version of the manuscript.

304 **Data availability**

305 All data generated or analyzed during this study are included in this article

306 **Competing Interests**

307 The authors declare no competing interests.

308 The author(s) declared that this work was conducted in the absence of any commercial or financial
309 relationships that could be construed as a potential conflict of interest.

310 **Funding**

311 This study was supported by Walter Bushnell Enterprises Pvt Ltd. Gurgaon, India through
312 a service agreement. The study also received material support in the form of study drugs and
313 antibiotics.

314 **Authors' Contribution**

315 All authors listed have made a substantial, direct, indirect, and intellectual contribution to the work,
316 and approved it for publication.

317 **Acknowledgements**

318 Not applicable

319 **References**

320 1. Vincent Ki, Coleman Rotstein. Bacterial skin and soft tissue infections in adults: A review of

their epidemiology, pathogenesis, diagnosis, treatment, and site of care. *Can J Infect Dis Med Microbiol.* 2008;19(2):173-84. doi: 10.1155/2008/846453.

2. Benjamin Silverberg. A Structured Approach to Skin and Soft Tissue Infections (SSTIs) in an Ambulatory Setting. *Clin. Pract.* 2021; 11(1): 65–74. doi: 10.3390/clinpract11010011.

3. Canton R, et al. Comparative *in vitro* antibacterial activity of ozenoxacin against Gram-positive clinical isolates. *Future Microbiology.* 2018; 13:3-19. Issue sup6
doi:10.2217/fmb-2017-0289.

4. Asha C. Bowen et al. The Global Epidemiology of Impetigo: A Systematic Review of the Population Prevalence of Impetigo and Pyoderma. *PLoS One*; doi: 10.1371/journal.pone.0136789. eCollection 2015.

5. Ray et al. Incidence, microbiology, and patient characteristics of skin and soft-tissue infections in a U.S. population: a retrospective population-based study. *BMC Infectious Diseases.* 2013; 13:252. <http://www.biomedcentral.com/1471-2334/13/252>.

6. Elisa Barbieri, et al. Non-bullous Impetigo: Incidence, Prevalence, and Treatment in the Pediatric Primary Care Setting in Italy. *Frontiers in Pediatrics.* 2022; Sec. Pediatric Infectious Diseases. Volume 10. doi: 10.3389/fped.2022.753694.

7. Bellis, K.L.; Dissanayake, O.M.; Harrison, E.M.; Aggarwal, D. Community Methicillin-Resistant *Staphylococcus aureus* Outbreaks in Areas of Low Prevalence. *Clin. Microbiol. Infect.* 2025; 31(2):182–189. doi: 10.1016/j.cmi.2024.06.006.

8. Ian Morrissey, Rafael Canton, Jordi Vila, Domingo Gargallo-Viola, Ilonka Zsolt, Maria Garcia-Castillo & Yuly Lopez. Microbiological profile of ozenoxacin. *Future Microbiology.* 2019; 14:773–787. doi: 10.2217/fmb-2019-0089.

9. Dadashi M, Hajikhani B, Darban-Sarokhalil D, Van Belkum A, Goudarzi M. Mupirocin resistance in *Staphylococcus aureus*: A systematic review and meta- analysis. *J Glob Antimicrob Resist.* 2020; 20:238–247. doi: 10.1016/j.jgar.2019.07.032.

10. Howden BP, Grayson ML. Dumb and dumber—the potential waste of a useful anti staphylococcal agent: emerging fusidic acid resistance in *Staphylococcus aureus*. *Clin Infect Dis.* 2006;42(3):394–400. doi: 10.1086/499365.

11. Ghea Aprillia, Ilham Setya Wicaksono. Treatment Strategies: A Systematic Review of The Advancements of Topical Antibiotics for Impetigo. *Journal Sehat Indonesia.* 2025; 7(2): 938.

12. Barman T.K., Kumar M, Chaira T., et al. Novel fluorobenzothiazole as a dual inhibitor of gyrase B and topoisomerase IV against Gram-positive pathogens. *Future Microbiol.* 2023; 18:625-638. doi: 10.2217/fmb-2022-0207. Epub 2023 Jun 22.

13. Jordi Vila, Adelaide A Hebert, Antonio Torrelo, Yuly López, Marta Tato, María García-Castillo and Rafael Cantón. Ozenoxacin: a review of preclinical and clinical efficacy. *Expert Review of*

356 Anti-Infective Therapy. 2019; 17, (3):159–168. <https://doi.org/10.1080/14787210.2019.1573671>.
357 14. Manisha Sahu, Vishal Dubey, Alka Maral. Ozenoxacin: A Comprehensive Review of its
358 Pharmacological Properties, Clinical Efficacy, and Emerging Applications in the Treatment of
359 Bacterial Skin Infections. International Journal of Pharmaceutical Research and Applications. 2023;8(5):901-
360 911. doi: 10.35629/7781-0805901911.